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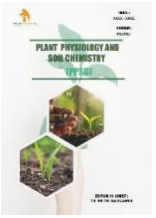

RESEARCH ARTICLE

EVALUATION OF CULTURE MEDIA FOR THE GROWTH OF *ALTERNARIA BRASSICICOLA*

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ABSTRACT

Alternaria leaf spot is one of the devastating diseases of cabbage caused by *Alternaria brassicola*. The experiment was conducted to understand the most effective culture media for the growth and multiplication of disease pathogens. Eight different culture media and four replications of each were employed under aseptic conditions for 12 days. Potato Dextrose Agar showed the highest radial growth (88.90mm) with a brownish to greenish colony color followed by Malt Extract Agar (87.45mm) with a whitish irregular margin of the dark brown colony. Sabouraud Dextrose Agar and Corn Meal Agar showed regular margins in their colony whereas Oat Meal Agar and Potato sucrose Agar showed irregular ones. Carrot Agar showed the lowest radial growth (53.12mm) with dark brown colony color at the center and dull reddish brown towards the margin which was followed by Nutrient Agar (59.62mm). This study will be a guide for further research on the physiology, taxonomy, and management of disease pathogens.

KEYWORDS

Alternaria Brassicola, Colony, Media Culture, Malt Extract Agar, Sabouraud Dextrose Agar

1. INTRODUCTION

Cabbage (*Brassica oleraceae var. capitata L.*) is a Brassicaceae vegetable with a leafy head. This family also includes cauliflower, broccoli, kale, Brussels sprouts, mustards, and other cold-time, resilient crops that grow best in chilly, wet conditions. In Nepal, the area cultivated is 28,530 ha and the production is 4,69,726 MT (MOALD, 2022). Cabbage thrives in a temperate, humid environment with moderate to high rainfall. It can withstand frost in the head stage, but cold conditions are detrimental to the rest of the plant. Cabbage can be grown in all soil types containing essential nutrients. For growing cabbage, a pH range of 6.0-6.5 is recommended and for crop development and head formation, temperatures between 15°C and 21°C are considered ideal. Flowering intensity is determined by the plants' age and the length of time they are exposed to cold temperatures (Bhattacharya et al., 1980).

Different biotic and abiotic stress affects the production of cabbage in terms of either quality or quantity. Alternaria Leaf spot in cabbage is a type of biotic stress caused by different *Alternaria spp.* Leaf spots, stunting, and damping-off are among the symptoms that may appear on young plants in seedbeds. Leaf spots caused by *A. brassicicola* start as tiny, black spots that quickly develop into circular lesions up to 1 cm in diameter (Pun et al., 2020). *Alternaria brassicicola* is a simple parasite that lives outside of its host saprophytically. The fungus is mainly transmitted via seeds, although it may also be transmitted through agricultural waste. Wind, water splash, humans, tools, and equipment all disseminate spores.

Alternaria brassicicola requires specific compounds for their growth and multiplications. Wide range of culture media needs for the radial growth of fungus, mycelial dry weights along with the characterization of sporulation. Morphological characteristic of fungus is major distinguishing features of fungus. Different fungi respond differently to a particular compound present in media (Shyama Sundar, 2015). In plants, Carbohydrates are present in simple and complex forms. Most fungi convert the complex form of carbohydrates into simpler water-soluble forms and utilize them. Without high-quality media, the chances of attaining precise growth and performance are low. If the media isn't flawless, the whole study may fail. Some studies have failed in the past owing to a lack of understanding of how Alternaria really appear and how quickly they may proliferate in different mediums. In the lab or elsewhere, media culture offers all of Alternaria's features, allowing researchers to provide treatment afterward. It's important to understand the colony features and growth performances of Alternaria before setting disease management strategies. This study will be a guide for further research on the Alternaria leaf spot caused by many Alternaria spp.

2. MATERIALS AND METHODS

The different culture mediums (solid) were evaluated for obtaining maximum mycelial growth of *Alternaria brassicicola*. The experiment was conducted in a complete randomized design with replicated 4 times. 8 solid media viz., Potato Dextrose Agar, Oatmeal Agar, Corn Meal Agar, Malt Extract Agar, Potato Sucrose Agar, Sabouraud Dextrose Agar, Carrot Dextrose Agar, Nutrient Agar. The culture mediums were prepared by the standardized method and autoclaved at 121.6°C, 15 psi pressure for 20 minutes. All of the experiments, isolations, subcultures and other investigations were carried out in a laminar airflow chamber under aseptic conditions. The chamber was disinfected using a UV light for fifteen minutes and ethanol wipes on the walls and base (70 %). By heating over a spirit lamp, the blades, forceps, inoculation loop, inoculation needle, and other instruments were disinfected.

After autoclaving, all of the Petri plates, prepared medium, and required equipment were transferred to laminar flow. As per the treatment and

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replication, 20 ml of each medium was put onto a 90mm sterile Petri plate and allowed to solidify. By cutting with a sterilized cork borer of 5mm inner diameter, a 5mm mycelial disc from a 10-day-old culture was inoculated at the center of a Petri plate and incubated at 27±1 °C for twelve days. The colony diameter was measured after 4, 8, and 12 days of inoculation, the diameter of the colony was measured at two positions at right angles to each other, and the average of the cross diameters was used to calculate the fungus' growth. At the time of the observations, sporulation was also observed.

3. Statistical Analysis

Ms. Excel (2013) was used to input all of the data. The studies were carried out in a lab setting, and the results were analyzed using SPSS statistical software and R studio utilizing a Completely Randomised Design (CRD). The Least Significance Difference (LSD) test was used to compare the means at the 0.05 level of significance.

4. Results

4.1 Radial Growth on Eight Different Media

A. brassicicola was allowed to grow in 8 different media to examine radial growth, as shown in Figure 1 and Table 2. As shown in Table 3, On the final day, Potato dextrose agar had the highest growth (88.90mm) with a dark green-brown center and white margin colony, followed by Malt extract agar (87.45mm) and Oat Meal Agar (86.52mm) with a dark brown center and green to brown colony margin. Corn Meal Agar with good sporulation and Sabouraud dextrose agar each had radial growth of (84.77mm) and (80.52mm), respectively. In carrot dextrose agar, the lowest growth was observed at 53.12mm. The nutrient agar medium showed no sporulation. As shown in Table 3, good sporulation was observed in Potato sucrose agar, Sabouraud dextrose agar, and carrot dextrose agar, whereas poor sporulation was observed in Oat Meal Agar.

4.2 Sporulation and Colony Characters of *A. Brassicicola* on Different Culture Media

Table 1: Colony character of *A. brassicicola* on different cultural media

S.N.	Culture Media	Colony Characters		
		Colony Colour	Sporulation	Margin of colony
1.	Corn Meal Agar	Dark brown to dark green	++	Regular
2.	Oat Meal Agar	Dark brown at the center and green to brown at the margin	++++	irregular
3.	Malt Extract Agar	Brown to dark brown at the center and whitish margin	++	Irregular
4.	Potato Dextrose Agar	Dark green-brown center and whitish margin	++	Regular
5.	Potato Sucrose Agar	Brownish center greenish brown at the outer side and a whitish margin	+++	Irregular
6.	Nutrient Agar	Whitish, more white center	+	Smooth irregular
7.	Sabouraud Dextrose Agar	Fully white thoroughly	+++	Regular
8.	Carrot Dextrose Agar	Dark brown at the center and dull reddish brown at the margin	++	Irregular

[(31-40) ++++ = Excellent, (21-30) +++ =Good, (11-20)++ =Moderate, (1-10) + = Poor)]

In the PDA medium, *A. brassicicola* showed moderate spores per microscopic field (11-20). In Malt Extract Agar, the spore population was relatively the same as in PDA media (11-20). Excellent sporulation was observed in Oat Meal Agar (31-40) spore per microscopic field as described in Table 1.

4.3 Evaluation of Media

There was a significant ($P \leq 0.05$) difference between using carrot dextrose agar and nutrient agar among Potato Dextrose Agar, Corn Meal Agar, Oat Meal Agar, malt extract agar, Potato sucrose agar, and Sabouraud dextrose agar. There was no significant difference in using Oat Meal Agar, Malt extract agar, Corn Meal Agar, and Potato dextrose agar. There was no significant difference between Potato sucrose agar and Sabouraud dextrose agar. Potato dextrose agar was non-significant with Malt extract agar and Oat Meal Agar. These four media also were nonsignificant with each other statistically. Similarly, Potato dextrose agar was non-significant ($P \leq 0.05$) between malt extract agar, Oat Meal Agar, corn meal agar, and sabouraud dextrose agar. But there was a significant ($P \leq 0.05$) difference between Potato sucrose agar and Potato dextrose agar as described in Table 2.

Table 2: Growth of *A. Brassicicola* on Different Culture Media

S.N.	Culture Media	Radial Growth at 12 days
1.	Carrot dextrose agar	53.12a
2.	Nutrient agar	59.62b
3.	Potato sucrose agar	78.00c
4.	Sabouraud dextrose agar	80.52c
5.	Corn Meal Agar	84.77d
6.	Oat Meal Agar	86.52de
7.	Malt extract agar	87.45de
8.	Potato dextrose agar	88.90e
	Grand mean	77.36
	SEm	2.379
	CV (%)	1.827
	LSD at 5%	2.063

5. Discussion

The investigation reveals that potato dextrose agar medium influenced the best radial growth of 88.90mm followed by malt extract agar in which a radial diameter of 87.45mm was observed. There was no huge difference between using potato dextrose agar and malt extract agar in terms of radial growth (Kumar et al., 2019). The best vegetative growth of Alternaria brassicicola was found in Potato dextrose agar which is supported by (Nur-E-Nasreen et al., 2017). A similar result was recorded in the findings of (Meena and Ratnoo, 2013). According to some study, the growth of Alternaria was found highest in Potato dextrose agar medium (Shyama, 2015; Sharma et al., 2018). According to Jain, 2018, the highest radial growth of *Alternaria spp* is in potato dextrose agar. The most fungus will thrive in the PDA medium in terms of radial growth because PDA has a complex carbon source from potato infusion also available in the easily utilizable form of carbon source, dextrose.

Nitrogen and other essential nutrients are provided by potato infusion. Potato Dextrose Agar contains complex carbon sources in easily utilizable form along with nitrogen sources and micronutrients. Besides mycelial diameter, Oat Meal Agar was found to have the highest spore population among 8 different culture media. Moderate sporulation was found in the PDA medium and Malt extract medium which might be due to the presence of a small amount of sucrose in malt extract and a relatively equal amount of all glucose, fructose, and sucrose in the PDA medium. Oat Meal Agar is less nutritious than all studied culture media so excellent sporulation was observed. Starvation or nutritional depletion often stimulates sporulation (Su et al., 2012). Too much nutrient leads to loss of sporulation.

6. Conclusion

Alternaria leaf spot is a severe disease that affects the production of cruciferous vegetables throughout the globe. It has a significant impact on crop quality and quantity. To develop appropriate disease management strategies, a thorough understanding of the influence of various culture media on fungus growth as well as sporulation and colony characteristics of the fungus is required. This research shows that the Potato Dextrose Agar medium showed the best radial growth with dark brown to the greenish colony with a white regular margin followed by Malt Extract Agar with brown to dark brown at the center and a whitish irregular margin. The least radial growth was observed in Corn Meal Agar with dark brown at the center and dull reddish-brown at the margin followed by Nutrient

Agar. Oat Meal Agar medium showed excellent sporulation whereas Potato Dextrose Agar medium showed moderate sporulation of *A. brassicicola.* Such a study may contribute to understanding the growing behavior, colony characteristics, and nature of *A. brassicicola.* The finding from the experiment will assist the interested researcher who intends to research *A. brassicicola* in the future. This study will be a guide for further research on the Alternaria leaf spot caused by many Alternaria spp.

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